ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1984, p. 283–288
0066-4804/84/090283-06$02.00/0

Vol. 26, No. 3

Effects of 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine, a New Antiherpesvirus Compound, on Synthesis of Macromolecules in Herpes Simplex Virus-Infected Cells

YUNG-CHI CHENG,* SUSAN P. GRILL, GINGER E. DUTSCHMAN, KARL B. FRANK, JWO-FARN CHIOU, KENNETH F. BASTOW, AND KOJI NAKAYAMA

Department of Pharmacology and Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

Received 25 February 1984/Accepted 12 June 1984

We examined the effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) on viral DNA, RNA, protein, and enzyme synthesis in HeLa cells infected with herpes simplex virus type 1 and type 2. DHPG inhibited virus DNA synthesis in a dose-dependent fashion. This inhibition was not due to the lack of deoxynucleoside triphosphates which are required for DNA synthesis. This compound has no apparent effect on early and late viral RNA synthesis, viral protein synthesis, or viral thymidine kinase, DNA polymerase, and DNase induction in virus-infected cells.

In recent years, several nucleoside analogs have been found to have selective antiherpesvirus activities. Among these, a new guanosine analog, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), was shown to have a broad and unique spectrum of antiherpesvirus activity at concentrations which were not toxic to several mammalian cells in culture (1, 4, 15, 20, 23, 24). Its antiviral activity in vivo was also demonstrated (8, 16, 23). In herpes simplex virus (HSV)-infected cells, DHPG was phosphorylated to DHPG monophosphate (DHPGMP) by virus-induced thymidine kinase (1, 4, 23) and further phosphorylated to DHPG diphosphate (DHPGDP) and DHPG triphosphate (DHPGTP) by cellular kinases (2, 8). DHPGTP could substitute for dGTP and be incorporated into DNA by virus-induced DNA polymerase in an efficient manner. The incorporation of DHPG could slow down DNA chain elongation. Furthermore, DHPG-terminated DNA could act as a potent inhibitor of virus-induced DNA polymerase by competing with activated DNA template (9). Early studies by other investigators indicated that DHPG inhibited viral DNA synthesis (23), but it was unclear whether DHPG had a selective effect upon the synthesis of other viral macromolecules such as protein or RNA. With the aid of newly developed techniques, we examined the effects of DHPG on viral macromolecule synthesis, and our results are reported in this communication.

MATERIALS AND METHODS

DHPG and [^{3}H]DHPG were kindly provided by Syntex (USA) Inc., Palo Alto, Calif. All other radioactive materials were purchased from ICN Pharmaceuticals, Inc., Irvine, Calif. Deoxynucleosides and deoxynucleotides were purchased from P. L. Biochemicals, Inc., Milwaukee, Wis. RPMI 1640 medium, Joklik modified medium, fetal calf serum, and horse serum were purchased from GIBCO Laboratories, Grand Island, N.Y. DNA polymerase (*Escherichia coli*) was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Nitrocellulose paper (BA85, 0.45 μm) was purchased from Schleicher & Schuell, Inc., Keene, N.H. HSV type 1 (HSV-1) and type 2 (HSV-2) antisera were purchased from DAKO Immunoglobulins, Santa Barbara, Calif. All chemicals used were reagent grade or better.

Cells and virus. Cells were grown at 37°C in RPMI 1640 medium containing 100 μg of kanamycin per ml and supplemented with 5% horse serum or 5% fetal calf serum for HeLa S_3 or Vero cells, respectively. All cultures were found to be mycoplasma free when tested by the 4,6-diamidino-2-phenylindole fluorescence technique (21). HSV-1 (strain KOS) and HSV-2 (strain 333) were maintained as previously described (3).

Preparation of samples for isopycnic gradient centrifugation. HeLa S_3 cells were plated in a six-well dish at 10^6 cells per 10-cm^2 well. Once the cells were attached to the dish, the growth medium was removed and replaced with Joklik modified (low-phosphate) medium containing 1% fetal calf serum. HSV-1 (KOS) was added at 3 PFU per cell and incubated at 37°C for a 1-h adsorption period. The medium containing virus was then removed, and the cell layer was washed with phosphate-buffered saline (PBS) (0.14 M NaCl, 4.0 mM KCl, 0.5 mM Na_2HPO_4, 0.15 M KH_2PO_4) and replaced with 1 ml of medium containing various concentrations of DHPG. After a 2-h incubation period, 25 μCi of $^{32}PO_4$ was added to each well. At 8 h after virus infection the samples were harvested by scraping the cells into the medium and centrifuging at 2,000 rpm for 10 min. The cell pellets were washed two times in cold PBS and lysed overnight in buffer (10 mM Tris hydrochloride, pH 7.9, 1 mM EDTA, 100 mM NaCl, 0.5% Sarkosyl, and 1% sodium dodecyl sulfate [SDS]) containing 1 mg of proteinase K per ml. This was followed by isopycnic gradient centrifugation as previously described (22).

Preparation of cytoplasmic RNA. HeLa S_3 cells were infected with HSV-1 (KOS) at 5 PFU per cell. After incubation for 1 h the virus was removed and the cell layer was washed with PBS. Growth medium was added to each flask with or without DHPG (5 μM). One treated and one control flask were harvested at 0, 3, and 6 h after viral infection. A rubber policeman was used to scrape the cell layer into the medium, and the cells were pelleted by centrifugation at 2,000 rpm. All subsequent steps were performed on ice with sterile equipment, glassware, and reagents to prevent enzymatic breakdown of the RNA. The cell pellet was resuspended in ice-cold PBS and recentrifuged. After decanting

* Corresponding author.

the supernatant, each pellet (approximately 0.1 ml) was suspended in 3.5 ml of PB_{1000} buffer (2.5 mM Tris hydrochloride, pH 7.1, 2.5 mM NaCl, 1 mM $MgCl_2$, 5% sucrose) containing 1 mg of sodium heparin per ml and homogenized in a Dounce homogenizer by six strokes with a B-type pestle. The homogenate was transferred to a Corex tube, mixed with 0.1 volume of Triton-DOC solution (10% Triton X-100 + 10% sodium deoxycholate), and centrifuged at 10,000 rpm for 10 min. The supernatant containing the cytoplasmic RNA was deproteinated at 25°C by shaking for 15 min with 0.1 volume of 10× SET buffer (100 mM Tris hydrochloride, pH 7.1, 50 mM EDTA, 10% [wt/vol] SDS) 0.5 volume of phenol, and 0.5 volume of chloroform. After centrifugation at 10,000 rpm for 15 min the aqueous phase was removed and brought to a final concentration of 0.2 M sodium acetate (pH 5.2). Two volumes of cold 100% ethanol was added, and the solution was kept at −20°C overnight and then centrifuged. The RNA pellet was suspended in water, and the solution was adjusted to a final concentration of 3 M sodium acetate (pH 6.0) and kept at 4°C for 40 min to precipitate the mRNA and rRNA (K. F. Bastow, R. Prabhu, and Y.-C. Cheng, Adv. Enzyme Regul., in press). The supernatant was discarded after centrifugation, and the RNA pellet was redissolved in water, followed by dilution with 2 volumes of 100% ethanol. RNA was precipitated overnight at −20°C, pelleted by centrifugation at 10,000 rpm for 10 min, and then redissolved in water. The concentration of RNA in the solution was determined by absorbance at 260 nm (24 absorbance units per mg of RNA) and adjusted to a final concentration of 1,000 μg/ml.

Agarose gel electrophoresis. A 1% agarose gel containing 6% Formalin and 1 μg of ethidium bromide per ml in running buffer (20 mM morpholinepropanesulfonic acid, 5 mM sodium acetate, pH 6.0, 1 mM EDTA, pH 7.0) was electrophoresed for 6 h at 80 V.

Preparation of samples for the agarose gel. Each sample contained approximately 5 μg of RNA in 5 μl of water, 2.5 μl of 10× running buffer, 12.5 μl of formamide, and 5 μl of 30% Formalin. The samples were heated to 65°C for 10 min and then cooled rapidly on ice. Before loading onto the gel, 12.5 μl of a solution of 50% sucrose and 0.4% bromophenol blue was added.

Transfer of RNA to nitrocellulose paper. The RNA from the electrophoresed gel was transferred to nitrocellulose paper by a diffusion blotting method. The nitrocellulose paper was placed in 20× SSPE buffer (3 M NaCl, 10 mM $KHPO_4$, pH 7.0, 1 mM EDTA) and blotted overnight. After the RNA was blotted onto the paper it was dried for 15 min under infrared light and then baked for 2 h in vacuo at 80°C.

Preparation and nick translation of DNA containing viral DNA. A hybrid plasmid with the insertion of the HSV *tk* gene at the *Bam*HI site of *E. coli* pBR322 was kindly provided by S. Bachenheimer, who obtained the plasmids from W. Summers (7). This 3.5-kilobase (kb) *Bam*HI fragment could hybridize with at least one early mRNA (1.4 kb) coding for viral *tk* and two late viral mRNA species (3.3 and 4.6 kb) under the conditions we employed.

^{32}P-labeled DNA was prepared by nick translation (19) with [α-^{32}P]dCTP to a specific activity of 1×10^8 to 3×10^8 cmp/μg.

Hybridization. The nitrocellulose filters were prehybridized for 2 h at 65°C in 20 ml of 6× SSPE–0.5% SDS buffer in a sealed plastic bag. The filters were then hybridized with 5×10^5 cpm of ^{32}P-labeled DNA per ml, previously prepared by nick translation.

Enzyme induction. Monolayers of HeLa S_3 cells (5×10^6 cells) were infected at a multiplicity of 5 PFU per cell with HSV-1. After 1 h of adsorption, the inoculum was removed, and medium was added with or without 5 μM DHPG. After virus had been allowed to replicate for various times, the cells were scraped into the medium, pelleted by centrifugation at 2,000 rpm, and washed twice in PBS.

Cell pellets were disrupted by sonication in 500 μl of extraction buffer (300 mM KH_2PO_4, pH 7.5, 2 mM dithiothreitol, 1 mM EDTA, 1 mM PMSF, 0.2% Triton X-100, 10% glycerol). Protein extracts were examined for thymidine kinase, DNA polymerase, and DNase activity by previously published methods (11, 14, 17). Thymidine kinase assays were performed in the presence and absence of 40 μM 5-bromovinyl deoxyuridine, which produces 50% inhibition of virus thymidine kinase in this assay but has a negligible effect on host enzyme. This allowed the detection of virus-specific enzyme in the presence of host cell thymidine kinase. Other conditions of the assay were the same as previously described (14).

Protein electrophoresis and immunostaining. Samples containing 10 μg of protein were precipitated with 5% trichloroacetic acid and then subjected to electrophoresis in 8.5% SDS-polyacrylamide gels by the method of Laemmli and Favre (13). Gels were placed between two sheets of nitrocellulose paper, and protein was transferred by the method of

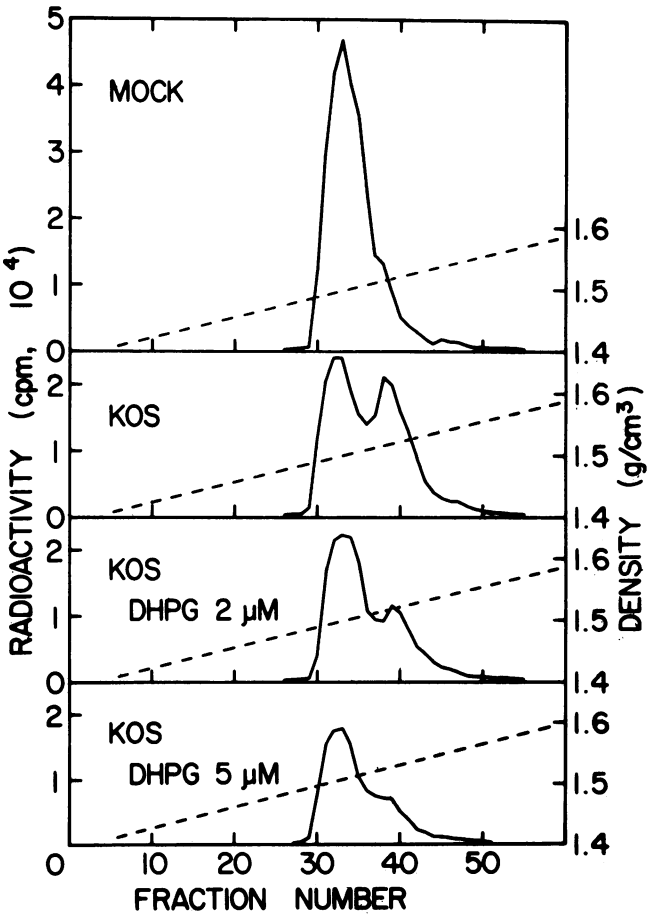

FIG. 1. Effect of DHPG on virus DNA synthesis. Isopycnic gradient centrifugation was performed on ^{32}P-labeled uninfected (Mock) and HSV-1-infected (KOS) HeLa S_3 cells treated with DHPG (0, 2, and 5 μM). Host DNA banded on the gradient at a density of 1.50 g/cm^3. Virus DNA banded on the gradient at a density of 1.52 g/cm^3. The details are described in the text.

TABLE 1. Effects of DHPG on deoxynucleoside triphosphate pool sizes in HSV-1-infected cells[a]

DHPG concn (μM)	Deoxynucleoside triphosphate (pmol)/10^6 cells)			
	dATP	dCTP	dTTP	dGTP + DHPGTP
0	38	12	60	12
1	34	39	175	39
5	39	41	165	53

[a] Details of the experiment are given in the text.

Towbin et al. (26). One sheet from each set was stained with amido black to reveal protein patterns and to show molecular weight markers. The other sheet was used to detect HSV-specific proteins by immunostaining procedures utilizing the technique previously described (6). The primary antibodies employed were prepared against lysates of HSV-1- and HSV-2-infected cells and were obtained from a commercial source. Utilizing this technique, only one protein band from mock-infected HeLa S_3 cells was detected. Virus-induced proteins appearing during the course of infection were correlated with those reported previously (12, 18).

Extraction and determination of deoxynucleoside triphosphates. HeLa S_3 cells (ca. 4×10^6) were seeded in a 25-cm^2 flask. Once the cells were attached, the growth medium was removed and replaced with HSV-1 (3 PFU per cell) for a 1-h adsorption period. The virus was then removed, and growth medium containing various concentrations of DHPG was added. After 6 h of exposure, the cells were collected, and deoxynucleoside triphosphate pool sizes were determined as previously described (27).

RESULTS

Effects of DHPG on DNA synthesis. HSV-1-infected cells were exposed to DHPG at various concentrations after a 1-h adsorption of virus. ^{32}P was added 2 h postinfection, and incubation was continued for 6 h. Cells were harvested and prepared for NaI isopycnic centrifugation as described above. The incorporation of radioactivity into viral DNA was inhibited more than that into host DNA (Fig. 1). The extracellular concentration of DHPG required to give 50% inhibition of ^{32}P incorporation into virus DNA was determined to be approximately 1 μM. An identical experiment was performed using HSV-2-infected cells, and similar results were obtained (data not shown).

Effects of DHPG on deoxynucleoside triphosphate pool sizes. Deoxynucleoside triphosphates were extracted from HSV-1-infected cells which were exposed to various concentrations of DHPG for 6 h postinfection. Exposure to DHPG did not result in a decrease of dATP, dCTP, or dTTP pool sizes (Table 1). Since determination of dGTP pool size could be affected by the presence of DHPGTP in cell extracts, this was investigated by adding 100 nM DHPGTP to known amounts of dGTP in the dGTP pool size assay employing *E. coli* DNA polymerase. The results indicated greater incorporation of ^3H label when DHPGTP was present (Table 2). When the data were plotted, two lines with identical slopes were obtained. The increment by which incorporation of ^3H label was increased by DHPGTP was equivalent to that produced by similar concentrations of dGTP. Therefore, the guanine nucleotide pools detected should be regarded as a combination of dGTP and DHPGTP pools.

Formation of DHPGTP in virus-infected cells. The amount of DHPGTP was determined in cells exposed to DHPG for 6 h after infection with HSV-1 or one of several phosphonoformic acid-resistant variants (Table 3). The amount of DHPGTP formed in cells infected by the parental virus was 9.1 pmol per 10^6 cells, which was similar to the value previously reported to us (2) and much less than those reported by other investigators (10, 25). This could be due to differences in the DHPG concentration employed, to different strains of virus or host cells, or to other factors. Cells infected with strain PFA^r-1 produced the greatest amount of DHPGTP (13.6 pmol per 10^6 cells), whereas those infected with PFA^r-5 produced the least DHPGTP (5.4 pmol per 10^6 cells).

TABLE 2. Increased [^3H]dTTP incorporation in the presence of DHPGTP during dGTP pool size determinations[a]

dGTP concn (nM)	cpm ± SD	
	Without DHPGTP	+ 100 nM DHPGTP
0	0 ± 202	1,422 ± 14
50	641 ± 68	3,005 ± 418
100	2,761 ± 13	2,909 ± 508
150	3,900 ± 62	5,122 ± 877
200	4,841 ± 101	6,802 ± 30
400	10,557 ± 711	11,886 ± 1252

[a] *E. coli* polymerase I was incubated at 37°C for 3.5 h with saturating amounts of dCTP, dATP, and [^3H]dTTP, utilizing calf thymus DNA in an assay as described previously (27). Various concentrations of dGTP were added with or without DHPGTP, as indicated. Results of one experiment are presented; the experiment was repeated several times, and similar results were obtained.

Viral RNA synthesis in DHPG-treated infected cells. Cytoplasmic RNA was extracted from HSV-infected cells 3 and 6 h postinfection with or without addition of 5 μM DHPG. It was possible to detect viral thymidine kinase and two late protein mRNA species by hybridization with a recombinant DNA probe which contained a HSV-1 DNA fragment. DHPG did not cause a drastic change in the amount of either thymidine kinase mRNA or late mRNA species (Fig. 2) as determined by densitometric scanning of the autoradiogram (data not shown).

Viral protein and enzyme induction in DHPG-treated cells. Proteins and enzymes were extracted from HSV-1- and HSV-2-infected cells with or without 5 μM DHPG treatment at different times postinfection. After SDS-gel electrophoresis, the proteins were transferred onto a sheet of nitrocellulose paper and immunostained with antiserum against HSV-1 and HSV-2 proteins. There was no apparent difference in the amounts and species of antibody-recognizable proteins between DHPG-treated and control infected cells (Fig. 3). Using HeLa S_3 as host cells, the induction of virus thymidine kinase, DNA polymerase, and DNase was examined. DHPG

TABLE 3. DHPGTP pools in cells infected with HSV-1 (KOS) or phosphonoformic acid-resistant variants[a]

Virus strain	DHPGTP concn (pmol/10^6 cells)
KOS	9.1
PFA^r-1	13.6
PFA^r-2	13.2
PFA^r-3	6.7
PFA^r-4	8.0
PFA^r-5	5.4

[a] HeLa S3 cells were infected at a multiplicity of 3 PFU per cell with HSV-1 (KOS) or one of five phosphonoformic acid-resistant variant strains (5). After exposure to 5 μM [^3H]DHPG (1.2 Ci/mmol) for 6 h, cells were harvested and extracted, and DHPGTP pools were determined by high-pressure liquid chromatography as described previously (2).

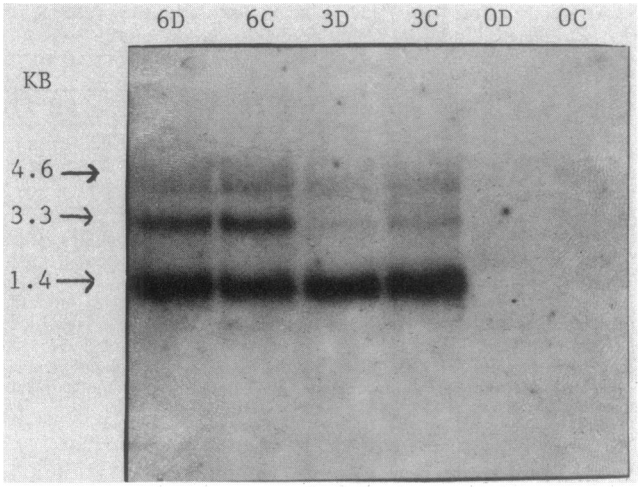

FIG. 2. Effect of DHPG on viral RNA synthesis in HSV-1-infected HeLa S_3 cells. The preparation of the RNA, agarose gel electrophoresis, blotting to nitrocellulose paper, nick translation, and hybridization are described in the text. DHPG was added to the growth medium, and samples were taken at 0, 3, and 6 h postinfection. C represents the RNA from control cells at the prescribed times. D represents the RNA from cells treated with 5 μM DHPG.

did not inhibit the induction of these three enzymes in virus-infected cells (Fig. 4).

DISCUSSION

DHPG could inhibit HSV DNA synthesis in infected cells in a dose-dependent fashion. This observation is consistent with those made by others (23). The concentration of extracellular DHPG required to inhibit 50% of viral DNA synthesis as measured by ^{32}P incorporation is higher than that reported when radioactive deoxynucleoside was employed as the precursor for estimating DNA synthesis. Whether this discrepancy is due to differences in the methodologies employed or the cell line and virus used is unclear. Our results are consistent with antiviral activity obtained for DHPG in yield reduction assays (4).

The inhibition of viral DNA synthesis in virus-infected cells could result from the lack of deoxynucleotides for DNA synthesis, the inhibition of the synthesis of mRNA and proteins which are required for DNA synthesis, or a direct action on the viral DNA synthesis apparatus. In examining the pool size of deoxynucleoside triphosphates, the results clearly demonstrate that there are sufficient deoxynucleoside triphosphates for DNA synthesis. There is an observed increase of some deoxynucleoside triphosphates which may relate to the inhibition of viral DNA synthesis in DHPG-treated cells. Thus the inhibition of viral DNA synthesis by DHPG could not be due to deoxynucleotide depletion as the result of the inhibition of some enzymes involved in deoxynucleotide formation. As a probe, a recombinant plasmid DNA containing a 3.5-kb virus DNA insert which could hybridize with three mRNA species in virus-infected cells was used, and the effect of DHPG on the synthesis of these three virus mRNA species was examined. DHPG had no drastic influence on the synthesis of either thymidine kinase mRNA (1.4 kb) or the other two late mRNA species. Thus, the general apparatus for the synthesis of virus mRNA does not appear to be inhibited by DHPG. Since the other virus mRNA species, which could not be detected by our probe, may be inhibited in a selective fashion and also the viral protein synthesis apparatus may involve guanosine nucleo-

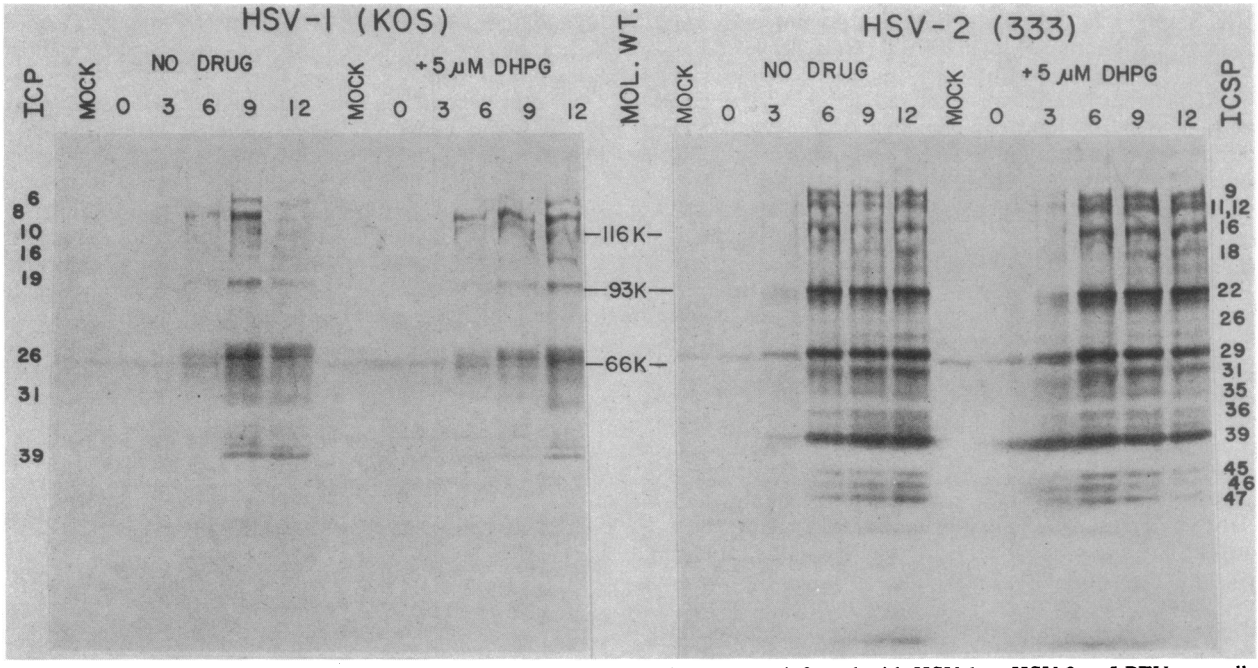

FIG. 3. Effect of DHPG on induction of viral proteins. HeLa S_3 monolayers were infected with HSV-1 or HSV-2 at 5 PFU per cell, and medium was added with or without 5 μM DHPG after a 1-h adsorption period. At various times postinfection cell monolayers were extracted as described in the text. Protein samples of 10 μg each were precipitated with 5% trichloroacetic acid and electrophoresed in 8.5% SDS gels along with molecular weight markers. Protein bands were blotted onto nitrocellulose sheets (26), and virus-specified proteins were detected by immunological staining (6). Infected cell protein (ICP) and infected cell specific protein (ICSP) numbers were assigned according to relative electrophoretic mobilities of protein bands reported in previous studies (12, 18).

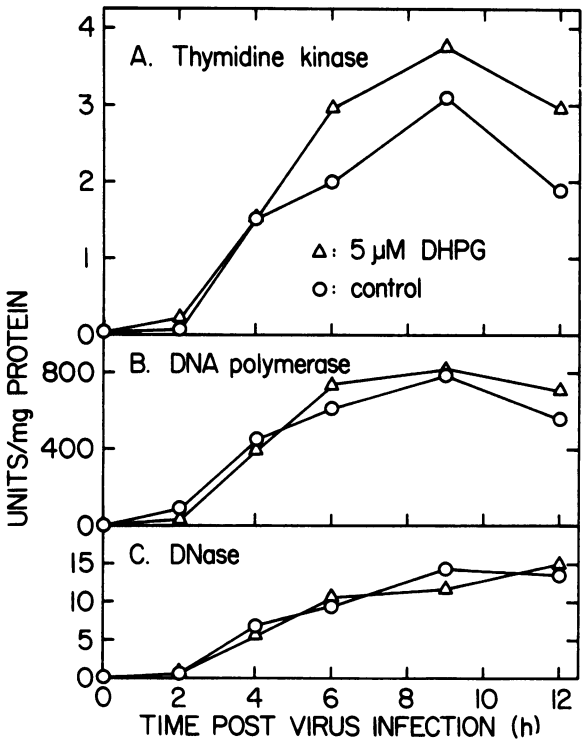

FIG. 4. Effect of DHPG on virus enzyme induction. HeLa S_3 monolayers were infected with HSV-1 at 5 PFU per cell. After a 1-h adsorption period, medium was added with or without 5 μM DHPG. At various times postinfection monolayers were scraped, washed, and examined for virus-induced thymidine kinase, DNA polymerase, and DNase activity as described in the text.

tide as do those of other mammalian cells, we examined the appearance of viral proteins, which are recognizable by antiserum, and the enzymes involved in viral DNA synthesis in infected cells treated with DHPG. No major difference in mock-treated and DHPG-treated cells was observed. This suggested that DHPG has no direct action on the viral protein synthesis apparatus and that DHPG is likely to have no effect on viral protein and RNA synthesis which may be required for viral DNA synthesis.

The antiviral action of DHPG is likely to be due to its action or that of its metabolites directly on the viral DNA synthesis apparatus in infected cells. Since viral DNA polymerase is a possible target, we have recently examined the effect of DHPGTP, a major metabolite of DHPG in infected cells, on the DNA chain elongation reaction catalyzed by HSV DNA polymerases isolated from cells infected by several strains of HSV. These virus variants have the same susceptibility to DHPG but different susceptibilities to acyclovir (4). There was a good correlation between the behavior of acyclovir triphosphate toward a viral DNA polymerase and the susceptibility of that virus to acyclovir (5), but no correlation between the behavior of DHPGTP toward a viral DNA polymerase and the susceptibility of that virus to DHPG (9). The uptake of DHPG and the formation of DHPGTP in cells infected by different virus variants were no more than threefold different (Table 3). This suggests that there are other reactions, in addition to the interaction of DHPGTP and viral DNA polymerase-catalyzed DNA chain elongation in the viral DNA synthesis apparatus, which may be partly responsible for the antiviral action of DHPG. We are now investigating such a possibility.

ACKNOWLEDGMENTS

This study was supported by a grant (CH-29) from the American Cancer Society and a gift from Syntex Co., Palo Alto, Calif.

LITERATURE CITED

1. **Ashton, W. T., J. D. Karkas, A. K. Field, and R. L. Tolman.** 1982. Activation by thymidine kinase and potent antiherpetic activity of 2'-nor-2'-deoxyguanosine (2'NDG). Biochem. Biophys. Res. Commun. **108**:1716–1721.
2. **Cheng, Y.-C., S. P. Grill, G. E. Dutschman, K. Nakayama, and K. F. Bastow.** 1983. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl) guanine, a new anti-herpes virus compound, in herpes simplex virus-infected cells. J. Biol. Chem. **258**:12460–12464.
3. **Cheng, Y.-C., S. Grill, J. Ruth, and D. E. Bergstrom.** 1980. Anti-herpes simplex virus and anti-human cell growth activity of *E*-5-propenyl-2'-deoxyuridine and the concept of selective protection in antivirus chemotherapy. Antimicrob. Agents Chemother. **18**:957–961.
4. **Cheng, Y.-C., E.-S. Huang, J.-C. Lin, E.-C. Mar, J. S. Pagano, G. E. Dutschman, and S. P. Grill.** 1983. Unique spectrum of activity of 9-[(1,3-dihydroxy-2-propoxy)methyl]-guanine against herpesviruses *in vitro* and its mode of action against herpes simplex virus type 1. Proc. Natl. Acad. Sci. U.S.A. **80**:2767–2770.
5. **Derse, D., K. F. Bastow, and Y.-C. Cheng.** 1982. Characterization of the DNA polymerases induced by a group of herpes simplex virus type 1 variants selected for growth in the presence of phosphonoformic acid. J. Biol. Chem. **257**:10251–10260.
6. **Domin, B. A., S. P. Grill, and Y.-C. Cheng.** 1983. Establishment of dihydrofolate reductase-increased human cell lines and relationship between dihydrofolate reductase levels and gene copy. Cancer Res. **43**:2155–2158.
7. **Enquist, L. W., G. F. VandeWoude, M. Wagner, J. R. Smiley, and W. C. Summers.** 1979. Construction and characterization of a recombinant plasmid encoding the gene for the thymidine kinase of herpes simplex type 1 virus. Gene **7**:335–342.
8. **Field, A. K., M. E. Davies, C. DeWitt, H. C. Perry, R. Lion, J. Germershausen, J. D. Karkas, W. T. Ashton, D. B. R. Johnston, and R. L. Tolman.** 1983. 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl] guanine: a selective inhibitor of herpes group virus replication. Proc. Natl. Acad. Sci. U.S.A. **80**:4139–4143.
9. **Frank, K. B., J.-F. Chiou, and Y.-C. Cheng.** 1984. Interaction of herpes simplex virus-induced DNA polymerase with 9-(1,3-dihydroxy-2-propoxymethyl) guanine triphosphate. J. Biol. Chem. **259**:1566–1569.
10. **Germershausen, J., R. Bostedor, A. K. Field, H. Perry, R. Liou, H. Ball, R. L. Tolman, and J. D. Karkas.** 1983. A comparison of the antiviral agents 2'-NOR-2'-deoxyguanosine and acyclovir: uptake and phosphorylation in tissue culture and kinetics of *in vitro* inhibition of viral and cellular DNA polymerases by their respective triphosphates. Biochem. Biophys. Res. Commun. **116**:360–367.
11. **Hoffman, P. H., and Y.-C. Cheng.** 1978. The deoxyribonuclease incuded after infection of KB cells by herpes simplex virus type 1 or 2. J. Biol. Chem. **253**:3557–3562.
12. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. J. Virol. **12**:1347–1365.
13. **Laemmli, U. K., and M. Favre.** 1971. Maturation of the head of bacteriophage T4. 1. DNA packaging events. J. Mol. Biol. **80**:575–599.
14. **Lee, L.-S., and Y.-C. Cheng.** 1976. Human thymidine kinase. I. Purification and general properties of the cytosol and mitochondria thymidine kinase derived from human acute myelocytic blast cells. J. Biol. Chem. **251**:2600–2604.
15. **Mar, E.-C., Y.-C. Cheng, and E.-S. Huang.** 1983. Effect of 9-(1,3-dihydroxy-2-propoxymethyl) guanine on human cytomegalovirus replication in vitro. Antimicrob. Agents Chemother. **24**:518–521.
16. **Martin, J. C., C. A. Dvorak, D. F. Smee, T. R. Matthews, and J. P. H. Verheyden.** 1983. 9-[(1,3-Dihydroxy-2-propoxy)methyl] guanine: a new potent and selective antiherpes agent. J. Med.

Chem. **26**:759–761.
17. **Ostrander, M., and Y.-C. Cheng.** 1980. Properties of herpes simplex virus type 1 and type 2 DNA polymerase. Biochim. Biophys. Acta **609**:232–245.
18. **Powell, K. L., and R. J. Coutney.** 1975. Polypeptides synthesized in herpes simplex virus type 2-infected HEp-2 cells. Virology **66**:217–228.
19. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase 1. J. Mol. Biol. **113**:237–251.
20. **Rollinson, E. A., and G. White.** 1983. Relative activities of acyclovir and BW759 against Aujesky's disease and equine rhinopneumonitis viruses. Antimicrob. Agents Chemother. **24**:221–226.
21. **Russell, W. C., C. Newman, and D. H. Williamson.** 1975. A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature (London) **253**:461–462.
22. **Ruth, J. L., and Y.-C. Cheng.** 1982. Selective antiviral agents. The metabolism of 5-propyl-2′-deoxyuridine and effects on DNA synthesis in herpes simplex virus type 1 infections. J. Biol. Chem. **257**:10261–10266.
23. **Smee, D. F., J. C. Martin, J. P. H. Verheyden, and T. R. Matthews.** 1983. Anti-herpesvirus activity of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine. Antimicrob. Agents Chemother. **23**:676–682.
24. **Smith, K. O., K. S. Galloway, W. L. Kennel, K. K. Ogilvie, and B. K. Radatus.** 1982. A new nucleoside analog, 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine, highly active in vitro against herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. **22**:55–61.
25. **St. Clair, M. H., W. H. Miller, R. L. Miller, C. U. Lambe, and P. A. Furman.** 1984. Inhibition of cellular α DNA polymerase and herpes simplex virus-induced DNA polymerases by the triphosphate of BW759U. Antimicrob. Agents Chemother. **25**:191–194.
26. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
27. **Williams, M. V., C. H. Chang, and Y.-C. Cheng.** 1979. An enzymatic method for distinguishing deoxyuridine and deoxythymidine nucleotide pools and its application for determining ribonucleotide reductase. J. Biomed. Biophys. Methods **1**:153–162.